

Evidence for Allelopathic Activity of *Mikania micrantha* H.B.K. on Three Weed Species

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ABSTRAK

Satu kajian makmal, rumah hijau dan lapangan telah dijalankan untuk menentukan pengaruh allelopati *Mikania micrantha* H.B.K. ke atas percambahan dan pertumbuhan tiga spesies rumpai iaitu *Asystasia gangetica* (L.) T. Anders., *Chrysopogon aciculatus* (Retz.) Trin dan *Paspalum conjugatum* Berg. Percambahan dan berat basah ketiga-tiga spesies berkurangan apabila kepekatan ekstrak daun dan akar *Mikania* semakin meningkat. Kesan fitotoksik ekstrak akar ke atas pemanjangan radikel spesies bioasai sangat jelas berbanding dengan ekstrak daun. Sarap *Mikania* (daun atau akar) yang diletakkan di atas permukaan atau digaul dalam tanah sangat mempengaruhi kemunculan dan berat basah spesies bioasai. Kemunculan *P. conjugatum* dipengaruhi oleh sarap daun atau akar *Mikania* yang digaul dalam tanah. Tanah yang diambil dari lapangan di mana ditumbuhi dengan *Mikania* didapati tidak menunjukkan kesan ke atas percambahan ketiga-tiga spesies bioasai. Bagaimanapun, berat kering *A. gangetica* (L.) T. Anders. dan *P. conjugatum* berkurangan secara bererti apabila ditanam dalam tanah yang telah ditumbuhi dengan *Mikania*.

ABSTRACT

Laboratory, greenhouse and field studies were conducted to determine the allelopathic potential of *Mikania micrantha* H.B.K. on the germination and growth of three weed species, *Asystasia gangetica* (L.) T. Anders., *Chrysopogon aciculatus* (Ritz.) Trin and *Paspalum conjugatum* Berg. Germination and fresh weight of all three weeds decreased progressively when exposed to increasing concentrations of aqueous leaf or root extract of *Mikania*. The phytotoxic effect of root extract on the radicle elongation of the test species was greater than that of leaf extract. The dry weight and rate of emergence of the bioassay species used were affected when *Mikania* debris (leaf or root) were placed on soil surfaces or incorporated into the soil. *P. conjugatum* emergence was strongly affected when *Mikania* (root or leaf) was incorporated into the soil. Soil collected from the field where *Mikania* had been growing had no effect on the final germination of the three weed species. However, dry weights of *A. gangetica* and *P. conjugatum* were significantly reduced when grown in the *Mikania*-infested soil.

Keywords: allelopathy, *Mikania micrantha*, debris, aqueous leaf extract, root extract, *Asystasia*, *Chrysopogon*, *Paspalum*

INTRODUCTION

The phenomenon of allelopathy has received increasing attention within the past two decades as a mechanism for explaining vegetation patterns in plant communities (Muller 1969; Rice 1979) and as an important aspect of weed-crop interactions (Tukey 1969; Whittaker 1970; Bell and Koeppel 1972). These interactions amongst plants often lead to a superiority of one species to the detriment of another under natural conditions.

Mikania micrantha, also known as mile-a-minute (ulam tikus), is listed as one of the world's worst weeds (Holm *et al.* 1977). It is a fast-growing, perennial creeping weed of the family Asteraceae and is widespread throughout Central America and the Asia-Pacific region. It has become a problem weed in cacao, rubber and oil-palm plantations in Malaysia (Watson *et al.* 1964; Holm *et al.* 1977). *Mikania* propagates by seeds and by rooting at stem nodes. Seeds are produced in large numbers on the masses of rampant climbing or creeping stems.

There is some evidence that the effects of *Mikania* on a crop may extend beyond the normal competition for nutrients, light and soil moisture. Lower nitrate-nitrogen levels were reported in soil planted with *Mikania* (Watson *et al.* 1964). The growth of rubber trees was affected when *Mikania* was the cover plant or when it had become dominant (Seth 1971). Wong (1964) reported that water extracts (1 and 2%) of oven-dried stem and leaf materials from *Mikania cordata* produced little growth response of tomato seedlings compared to water extracts of *Paspalum conjugatum* and *Pueraria phaseoloides*. Its extract also caused comparatively low nitrogen and phosphorus levels in test plants. Extracts of *Mikania cordata* significantly reduced nitrification in the incubation pot (Wong 1964). The inhibitory compounds were found to be phenolic and flavonoid substances.

Our field observations suggest that communities dominated by *Mikania* often have a reduced density of associated herbaceous species compared to adjacent areas. Therefore, the presence of this weed may influence growth of certain crops or weeds. Further studies are needed to identify the nature of inhibitors present in *Mikania* and to determine their residual activity under field conditions.

The objectives of this study were to evaluate potential allelopathic effects of *Mikania* by determination of 1) the phytotoxic effect of aqueous extract of leaves and roots, 2) the effect of soil-incorporated or surface-applied *Mikania* debris on test plants and 3) inhibitory effects of *Mikania*-infested field soil samples on germination and growth of three weed species, namely *Asystasia gangetica*, *Chrysopogon aciculatus* and *Paspalum conjugatum*.

MATERIALS AND METHODS

Plant Materials

Plant materials, including roots and leaves of *Mikania*, were collected for this study from rubber plantations near Sungai Buluh, Selangor, Malaysia. The

plants, at flowering stage, were collected in July and September 1991. Soil cores (10 cm diameter by 15 cm depth) were collected from the same location using a hand-held soil sampler. The soil type was a Sungai Buluh series, containing 86% sand, 4% silt and 10% clay with 1.31% organic carbon and pH 4.6. *A. gangetica*, *P. conjugatum* and *C. aciculatus* seeds were collected from the same area and used as bioassay species.

Effect of Aqueous Extracts of Leaves and Roots on Bioassay Species

These experiments were conducted to determine if any substance(s) in weed debris (roots or leaves) inhibited the growth of other plants. Ten g of fresh leaves and roots were cut separately into 2-4 cm lengths before extraction. Plant materials were kept in a flask containing 350 ml distilled water and agitated for 2 hours on an orbital shaker (140 rpm) at room temperature ($27 \pm 3^\circ\text{C}$). The extract was strained through four layers of cheesecloth and then through two layers of Whatman No. 2 filter paper to remove solid materials. Three concentrations of aqueous extract were used for the experiment, i.e. full-strength (28.60 g l^{-1}), half-strength (14.30 g l^{-1}) and quarter-strength (7.15 g l^{-1}). Dilutions were made with distilled water.

Twenty seeds each of *A. gangetica*, *P. conjugatum* and *C. aciculatus* were placed in separate petri dishes lined with 9-cm Whatman No. 2 filter paper. Ten ml of either extract, or distilled water for controls, were used to wet each filter paper. Five replicates were used for each concentration of each extract. The covered petri dishes were incubated at 30°C . The percentage germination, radicle length and fresh weight of the bioassay species were recorded after 7 days. Seeds were considered germinated when radicle length was over 2 mm. Radicle length and fresh weight of the seedlings were expressed as percentages of the distilled water control.

Effects of Leaf and Root Debris

These studies were conducted to determine effect of leaf and root debris on growth of test plants grown in a soil medium. Air-dried leaves and roots of *Mikania* were cut into 2-4 cm lengths and put separately into polyethylene bags containing the same type of soil. Five concentrations of leaf or root tissue (0, 1.5, 3, 6 or 12 g dry weight per 1800 g soil) were either placed on the soil surface or mixed thoroughly throughout the entire potting soil. The 6 g debris per 1800 g soil concentration was comparable to a field dry matter of 4900 kg ha^{-1} leaf or root (derivation based on $2.24 \times 10^6 \text{ kg soil/ha/15 cm depth}$). There were five replicates per treatment for each bioassay species.

Ten seeds of *A. gangetica*, *P. conjugatum* and *C. aciculatus* were each sown separately in polyethylene bags (8 cm \times 12 cm) and watered regularly at 80% field capacity. Seedling emergence was recorded 14 days after planting and the plants were thinned to two seedlings per bag. Plants were harvested 4 weeks after planting and dry weights were determined. Seedling dry weight data were converted to percentage of control.

Effect of Mikania-infested Soil on Bioassay Species

These studies were designed to determine whether soil infested with *Mikania* had an effect on the growth of other plants. Soil cores (10 cm diameter and 15 cm depth) were taken from soil infested with *Mikania* and placed in an 8 cm × 12 cm polyethylene bag. For control, soil cores were collected from the same field from areas which were free of plant debris. Hand-weeding was carried out frequently two months before collecting the control soil. The soil was sieved through 3 mm mesh before being put into the bag. The soils had not been treated previously with herbicides or other chemicals for about two years.

Ten seeds of each bioassay species were sown separately in polyethylene bags (containing 1800 g soil), 1 cm below the soil surface, and incubated in the greenhouse. There were five replicates of bioassay species for each soil sample. All polyethylene bags were watered regularly to maintain soil moisture at 80% field capacity. No artificial light was supplied; the temperature during the experimental period ranged between 28 and 34°C. Seedling emergence was recorded 14 days after planting, and plants were thinned to three seedlings per bag. The plants were harvested 4 weeks after planting and dry weights were determined.

Statistical Analysis

A complete randomized design with five replications was used for the debris and extract studies. Experiments of leaf/root debris and aqueous extract were conducted twice and means of ten replicates were averaged. Analysis of variance was performed on all data.

RESULTS*Effect of Aqueous Extracts of Leaves and Roots on Bioassay Species*

The full-strength leaf extract (28.6 g/l) reduced germination of the two bioassay species, *A. gangetica* and *P. conjugatum* by 60 and 43%, respectively

TABLE 1
Germination of bioassay species in the presence of leaf and root extract of *Mikania*

Extract conc. (g/l)	<i>A. gangetica</i>		<i>C. aciculatus</i>		<i>P. conjugatum</i>	
	Leaf	Root	Leaf	Root	Leaf	Root
% germination						
0.00	96 ^a	96 ^a	72 ^{ab}	72 ^a	86 ^a	86 ^a
7.15	55 ^b	56 ^b	81 ^a	56 ^{bc}	52 ^b	63 ^b
14.30	45 ^{bc}	39 ^c	74 ^{ab}	65 ^{ab}	44 ^b	59 ^b
28.60	36 ^c	34 ^c	66 ^b	46 ^c	43 ^b	59 ^b

Means followed by the same letter in each column are not significantly different ($p < 0.05$) as determined by Duncan's multiple range test.

(Table 1). Both species also showed significant reduction in germination even at the lowest concentration of 7.15 g/l of leaf extract. However, germination of *C. aciculatus* seed was less affected by the *Mikania* leaf extract, with no significant reduction in germination even at the highest concentration. At full strength, the extract of *Mikania* roots reduced the germination of *A. gangetica* and *P. conjugatum* by 62 and 27% respectively. Thus both leaf and root extracts reduced germination of *A. gangetica* and *P. conjugatum* more than *C. aciculatus*.

The results show that the root extract affected radicle length more than the leaf extract did, especially at higher concentrations. Full-strength aqueous leaf extract reduced radicle length of *A. gangetica*, *C. aciculatus* and *P. conjugatum* by 45, 39, and 21% of control, respectively (Table 2). Generally, the radicle length of all bioassay species decreased with increasing concentrations of *Mikania* root extract, with *A. gangetica* being the most susceptible to the root extract at full strength (Table 2).

TABLE 2
Radicle length of bioassay species when germinated in the presence of
leaf and root extract of *Mikania*

Extract conc. (g/l)	<i>A. gangetica</i>		<i>C. aciculatus</i>		<i>P. conjugatum</i>	
	Leaf	Root	Leaf	Root	Leaf	Root
	% of control					
0.00	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
7.15	99 ^a	79 ^b	80 ^b	84 ^b	84 ^b	90 ^a
14.30	93 ^a	57 ^c	62 ^c	71 ^b	81 ^b	69 ^b
28.60	55 ^b	29 ^d	61 ^c	42 ^c	79 ^b	34 ^c

Means followed by the same letter in each column are not significantly different ($p < 0.05$) as determined by Duncan's multiple range test.

The fresh weights of *A. gangetica*, *C. aciculatus* and *P. conjugatum* seedlings were reduced by 42, 47 and 29% of control, respectively, with full strength *Mikania* leaf extract (Table 3). At 28.6 g l⁻¹, the root extract caused greater reduction in the fresh weight of *A. gangetica* (56% of control) than in the other two species. At the same concentration, the fresh weights of *C. aciculatus* and *P. conjugatum* were reduced by 21 and 19% of the control, respectively.

Effects of Leaf and Root Debris

The emergence of *A. gangetica* was affected by increasing amounts of *Mikania* leaf debris incorporated into soil at 6 g per 1800 g soil (Fig. 1). When leaf debris were incorporated into soil at 12 g/1800 g, the emergence of *A. gangetica* declined by 38% of control. Root debris incorporated into soil showed a pattern of inhibition similar to that of leaf debris. The emergence of *A. gangetica* was found to show no correlation with the increasing amount of root

or leaf debris placed on the soil surface. Root debris on the soil surface reduced emergence by 32% of control at 1.5 g/1800 g soil, but emergence reached control levels at the highest concentration.

TABLE 3
Fresh weight of bioassay species when germinated in the presence of leaf and root extract of *Mikania*

Extract conc. (g/l)	<i>A. gangetica</i>		<i>C. aciculatus</i>		<i>P. conjugatum</i>	
	Leaf	Root	Leaf	Root	Leaf	Root
	% of control					
0.00	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
7.15	65 ^b	81 ^b	97 ^a	90 ^{ab}	79 ^b	96 ^a
14.30	58 ^b	47 ^c	78 ^b	89 ^{ab}	74 ^b	89 ^{ab}
28.60	58 ^b	44 ^c	53 ^b	79 ^b	71 ^b	81 ^b

Means followed by the same letter in each column are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

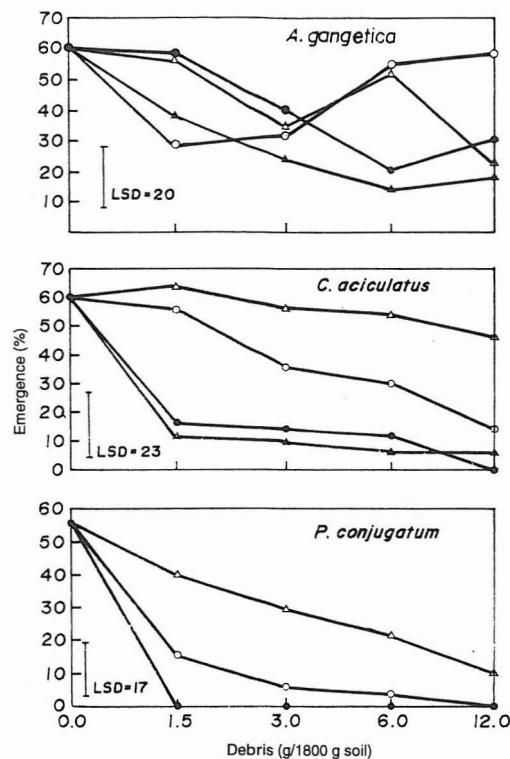


Fig. 1. Emergence of three bioassay species when germinated in the presence of root debris or leaf debris of *Mikania* either on soil surface or incorporated into soil (Δ leaf, soil surface; ○ root, soil surface; ▲ leaf, incorporated; ● root, incorporated)

Mikania root or leaf debris caused greater reduction of *C. aciculatus* emergence when incorporated in the soil at a rate of 1.5 g/1800 g soil (Fig. 1). The emergence was reduced to 48% and 44% when *Mikania* leaf or root debris were incorporated at 1.5 g/1800 g soil. At lower concentrations, leaf debris on the soil surface were less effective in reducing emergence of *C. aciculatus*. The emergence of *C. aciculatus* was reduced by only 12% of control at 12 g leaf/1800 g soil on the soil surface. On the other hand, root debris generally reduced germination of *C. aciculatus* with increasing rates of root debris on the soil surface.

No emergence of *P. conjugatum* seedlings was observed when leaf or root debris were incorporated into the soil. Emergence of *P. conjugatum* seedlings was reduced with increasing amounts of debris (root or leaf) on the soil surface. Significant differences in rates of emergence were observed between surface root debris and surface leaf debris at less than 6 g debris/1800 g soil.

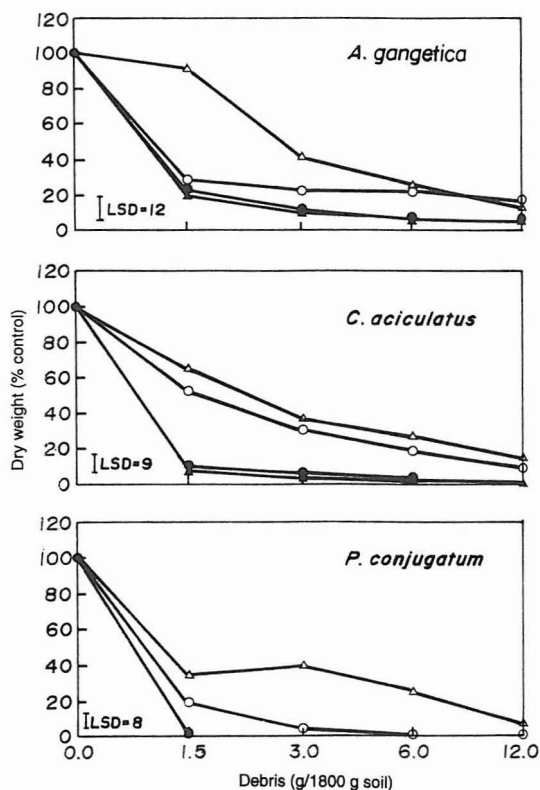


Fig. 2. Dry weight of three bioassay species when germinated in the presence of root debris or leaf debris of *Mikania*, either on soil surface or incorporated into the soil (△ leaf, soil surface; ○ root, soil surface; ▲ leaf, incorporated; ● root, incorporated)

The dry weight of *A. gangetica* was decreased by surface residues of either leaves or roots only at the highest rate (Fig. 2). The leaf debris had less effect on dry weight of *A. gangetica*, which was reduced to less than 20% of control

when leaf or root debris were incorporated into the soil at the rate of 1.5 g/1800 g soil.

The dry weight of *C. aciculatus* declined significantly with increasing concentrations of incorporated *Mikania* leaf or root debris (Fig. 2). Greater reduction of dry weight of *A. aciculatus* was observed when leaf or root debris were incorporated in the soil. When leaf or root debris at the highest rate were placed on the soil surface, the dry weight of *C. aciculatus* was reduced to 18% and 20%, respectively.

The results clearly show that the dry weight of *P. conjugatum* was also affected when either leaf or root debris of *Mikania* were incorporated into the soil (Fig. 2). The dry weight of *P. conjugatum* decreased with increasing levels of *Mikania* debris, falling to 6% of control when grown in soil with *Mikania* leaf debris on the surface at 12 g debris/1800 g soil. Dry weights of *P. conjugatum* were affected more by incorporated residues of *Mikania* than by residues present on the soil surface. In general, the dry weight of all three bioassay species was lower when grown in soil with either leaf or root debris incorporated in the soil than when debris were placed on the surface.

Effect of Mikania-infested Soils on Germination and Growth of Bioassay Species
Soil from beneath *Mikania* plants did not affect the final percentage of seed germination of any of the species tested (Table 4). However, seedling dry weights of *A. gangetica* and *P. conjugatum* were lower when seeds were germinated in *Mikania*-infested soil than when they were grown in soil collected away from *Mikania*. The results suggest that *Mikania* debris might produce, directly or indirectly, inhibitory effects on the growth of certain species.

TABLE 4
Germination and growth of bioassay species in *Mikania*-infested soil

Soil origin	<i>A. gangetica</i>		<i>C. aciculatus</i>		<i>P. conjugatum</i>	
	G (%)	DW (mg)	G (%)	DW (mg)	G (%)	DW (mg)
<i>Mikania</i> -infested soil	62 ^a	402 ^b	60 ^a	88 ^a	56 ^a	9 ^b
non-infested soil	63 ^a	671 ^a	61 ^a	88 ^a	60 ^a	55 ^a

DW = Dry weight/2 plants; G = Germination

Means followed by the same letter in each column are not significantly different ($p < 0.05$) as determined by Duncan's multiple range test.

DISCUSSION

The experiments described here provide evidence of the existence of inhibitory factors in *Mikania* which can influence germination and growth of certain weed species. The test species showed different responses to *Mikania* extract and to debris in soil, and also responded differently to root and leaf debris. Corn, cotton and morning glory have been reported to be similarly affected by the presence of debris of crimson clover (*Trifolium incarnatum* L.) and hairy vetch (*Vicia villosa* Roth) when incorporated in soil (White *et al.* 1989).

Quantities of allelochemicals vary between different plant tissues, and under different phenological and environmental conditions (Putnam and Duke 1978; Rice 1984). The magnitude of allelopathic interactions is also dependent upon the concentration and chemical stability of the active compounds as well as upon plant tolerance to such compounds and their microbial metabolites (Phillips *et al.* 1980). Thus, accurately characterizing allelopathy and its relative impact can be difficult unless such aspects are taken into account. Identification of the compound(s) responsible for the effects observed in these studies would further define this probable allelopathic interaction.

Test plants in the debris study responded not only to amount and type of debris (roots or leaves) but also to the change in location of debris. Thus location of debris in relation to growing roots appears to be an important factor in the allelopathic interaction (White *et al.* 1989). Toxicity of *Mikania* is enhanced when its debris are incorporated into the soil; the process of incorporation promotes its chemical and microbial decomposition, releasing potentially toxic soluble organic constituents. Weed or crop seeds and roots in close proximity would therefore have a greater probability of coming into contact with allelopathic compounds (Barnes and Putnam 1986). Most of the allelopathic compounds such as alkaloids, phenols and various coumarin derivatives are absorbed by roots (Winter 1961). The ineffectiveness of surface-applied debris in reducing seedling dry weight may be due to adsorption of leachates near the soil surface, allowing only limited contact with roots of emerging seedlings. Debris located on the soil surface may decompose at a slower rate and thus release allelochemicals in lower, easily-adsorbed quantities, distant from expanding roots (Achhireddy and Singh 1984). In addition, debris on the soil surface may provide better conditions (such as soil moisture and temperature) for soil microbial activities (Hance 1973). Earlier reports have shown that the number of soil microbes increase in soil amended with certain allelochemicals such as ferulic acid (Rice 1979). Some fungal species are able to use ferulic acid as a sole carbon source (Black and Dix 1976).

Root and leaf extracts exerted different levels of toxicity on the bioassay species. The root extract was more toxic to the growth of the three species than was leaf material. Similar observations were made by Achhireddy and Singh (1984), who found that lantana roots were more toxic on milkweed vine than its leaves. Our results have shown that root debris on the soil surface caused

greater reduction in the emergence rate and dry weight of *P. conjugatum* than did leaf debris. A possible explanation for this difference could be qualitative and/or quantitative differences in allelochemicals present in *Mikania* root tissue and leaf tissue.

Results of these studies suggest that *Mikania* has an allelopathic influence on other plants in addition to its competitive ability, as reported previously by Wong (1964). Verification rests both on isolation of one or more active compounds from plants and on evidence that they exist and act in the soil system.

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